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RESEARCH ARTICLE

Physiological flexibility and acclimation to food shortage in a heterothermic primate

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SUMMARY

As ecosystems undergo changes worldwide, physiological flexibility is likely to be an important adaptive response to increased climate instability. Extreme weather fluctuations impose energetical constraints such as unpredictable food shortage. We tested how grey mouse lemurs (*Microcebus murinus*) could adjust their daily heterothermy and locomotor activity to these 'energetic accidents' with a food restriction experiment. The experimental design consisted of acute calorie restriction (2 weeks, 80% restriction) in the middle of winter, after a fattening season with low (11 weeks, 40% restriction) *versus* high (*ad libitum*) food availability. This design aimed at simulating the combined effects of the quality of the fattening season (acclimation effect) and a sudden, severe food shortage during the lean season. Hour of start and duration of torpor were the most flexible components of energy savings, increasing in response to the acute food shortage with facilitation by chronic restriction (acclimation effect). Modulations of locomotor activity did not support the hypothesis of energy savings, as total locomotor activity was not reduced. Nonetheless, acutely restricted individuals modified their temporal pattern of locomotor activity according to former food availability. We provide the first experimental evidence of different temporal levels of flexibility of energy-saving mechanisms in a heterotherm exposed to food shortage. The acclimation effect of past food scarcity suggests that heterothermic organisms are better able to respond to unpredicted food scarcity during the lean season. The flexible control of energy expenditure conferred by heterothermy may facilitate the plastic response of heterothermic species to more frequent climatic hazards.

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Key words: climate change, calorie restriction, energy saving, extreme climatic event, food availability, *Microcebus murinus*, phenotypic plasticity, thermoregulation.

INTRODUCTION

As global environmental changes progress, understanding the mechanisms of adaptation to rapid environmental change is becoming a major research area in ecology (Stenseth et al., 2002; Walther et al., 2002). In recent decades, climate change has disturbed ocean thermohaline circulation (Broecker, 1997), impacting the timing and nature of El Niño events (Trenberth and Hoar, 1997) and North Atlantic Oscillations (Beniston and Jungo, 2002). These changes affect the frequency, duration, geography and/or severity of extreme events (large floods, droughts, tropical storms) likely to be prevailing in the tropics (Easterling et al., 2000; IPCC, 2007). As primary production is largely constrained by climatic conditions, extreme climatic events translate into disturbed seasonal patterns of food availability, such as food shortage, cascading on upper levels of the trophic chains (Stenseth et al., 2003; Durant et al., 2007).

Phenotypic plasticity would be the predominant adaptive response of vertebrates to ongoing rapid climate changes (Charmantier et al., 2008; Gienapp et al., 2008), potentially with selection for the most plastic genotypes (Nussey et al., 2005). Phenotypic flexibility is defined as the ability of individuals to rapidly modify their phenotype according to changes in environmental conditions. In highly unpredictable environments (Dewar and Richard, 2007), it is supposed that high physiological flexibility (intra-individual modulations of the physiological phenotype) has evolved in response to the heterogeneity and instability of environmental selective pressures. Indeed, in the tropics, resource availability is determined primarily by the magnitudes and frequencies of rainfall, which are subject to the El Niño Southern Oscillation (ENSO) in the Afrotropical zone (Stone et al., 1996). Predictable characteristics of food abundance are affected within the year by the wet and the dry seasons with a high interannual variability (La Niña or El Niño events). In these regions, the unpredictability of resource availability has selected for organisms that can compensate the high fluctuation of their environment by adjustments of their physiology (Hau et al., 2000; Lovegrove, 2000). However, the physiological mechanisms involved in the response to climate changes, and particularly extreme climatic events, are still poorly understood (Parmesan, 2006). If these climate changes are to be compensated for essentially by plastic adjustments, experimental biology could contribute to the understanding and forecasting of the impact of climate change by characterizing the extents, limits and/or facilitators of physiological flexibility (Naya et al., 2009).

In the present study, we are interested in identifying which physiological traits could be rapidly adjusted to compensate for an abrupt environmental change resulting in a food shortage and whether these adjustments benefit from acclimation. Our experimental design aimed at simulating the occurrence of an extreme climatic event during winter, such as an intense drought or a cyclone, leading to an acute food shortage, after a bad *versus* a good fattening season (i.e. a chronic food shortage *versus ad libitum* food availability, respectively). Contrasted food regimes,

with a previous exposure to a chronic food shortage, allow the quantification of the flexible modulations of physiological mechanisms. Crossing a chronic with an acute food shortage allows us to test for the effect of acclimation to food shortage. Acclimation is defined as the facilitation of the phenotypic response to an environmentally stressful change by former, long-lasting presentation to intermediate levels of this same stress (Bowler, 2005). If acclimation is effective, it suggests that a period of unfavorable conditions (e.g. low autumnal food availability) would facilitate the physiological compensation of an extreme event (e.g. severe winter drought).

Small mammals living in unpredictable environments are good models with which to investigate mechanisms of environmentally driven physiological flexibility. We investigated the physiological responses of a small heterothermic primate faced with contrasted regimes of food shortage. Small lemurs (Cheirogaleidae) are interesting in this regard because they evolved under the unpredictable climatic conditions of Madagascar (Dewar and Richard, 2007). This island is exposed to El Niño–La Niña climatic oscillations, whose frequency and intensity would be amplified by current climate change (Trenberth and Hoar, 1997). The grey mouse lemur (*Microcebus murinus*) is the most widespread species on the island, with populations established in the most seasonal and unstable habitat of the island: the occidental dry forest (Olivieri et al., 2007). The western region shows strikingly more interannual variability in rainfall than other regions of the island (Dewar and Richard, 2007). The resulting fluctuations of resource availability would have selected for the energy conservation mechanisms exhibited by mouse lemurs: fat storage, daily torpor during resting and reduction of locomotor activity (Wright, 1999; Schmid, 2001; Ganzhorn et al., 2003). Heterothermy is often associated with unpredictable environments, e.g. in austral, tropical and semitropical ecosystems (Lovegrove, 2000; Grigg et al., 2004; Geiser, 2004b). It has evolved in these regions in response to the unpredictable spatial flushes of resource to offset the energetic costs of normothermia maintenance. In grey mouse lemurs, torpor expression and the decrease of locomotor activity would be adaptations to the seasonal food and/or water shortages, maximizing survival and/or subsequent reproductive output (Schmid and Speakman, 2000; Schmid, 2001; Radespiel, 2006; Rasoazanabary, 2006). Daily torpor is the periodic reduction of body temperature resulting from the daily metabolic rate being shut off when entering the resting phase (Heldmaier and Ruf, 1992; Geiser and Ruf, 1995). Body temperature of animal is a suitable indicator of torpor use as it is correlated to the metabolic rate (Schmid et al., 2000; Schmid and Speakman, 2000). A 76% reduction of the metabolic rate during torpor in the grey mouse lemur induced an important decrease of energy expenditure (~38%) (Schmid, 2000). Rather than facultative hypothermia, which is an emergency survival strategy (Tomlinson et al., 2007), torpor would be a routine energy conservation strategy for grey mouse lemurs during the dry season (Schmid, 2000). Experimental studies of calorie restriction revealed an adjustment in time and intensity of daily heterothermy according to the length and intensity of the food shortage (Genin and Perret, 2003; Séguy and Perret, 2005; Giroud et al., 2008). Concerning locomotor activity, there is an increase in total activity during the first days of restriction (Genin and Perret, 2003), with a concentration in the first hours of the night (Giroud et al., 2008).

Season, body fat reserves, food availability and ambient temperature influence the use of torpor (Geiser, 2004a). The present study provides the first experimental test of the effect of acclimation to calorie restriction on the levels of physiological flexibility that a heterothermic mammal can express in response to a sudden food shortage. The physiological components of the flexibility of energy savings that are characterized are daily heterothermy and locomotor activity. Physiological modifications within days in response to an acute food shortage would indicate a high physiological flexibility (rapid adjustment), whereas response to the chronic restriction only would be indicative of lower flexibility (delayed adjustment). Facilitation of the response to an acute food shortage by a previous exposure to moderate food shortage would demonstrate an acclimation effect. Finally, no response to food shortage would indicate an absence of flexibility. The effectiveness of calorie restriction treatments is quantified by changes in food efficiency. We expect that daily heterothermy will be increased in response to calorie restriction, but with different levels of flexibility for the different temporal components of torpor (time of body temperature decrease and of minimum body temperature, and duration and depth of torpor). The locomotor activity should be reduced according to the intensity of calorie restriction. If there is acclimation to calorie restriction, we expect previously calorie-restricted individuals to exhibit a higher physiological flexibility than those fed *ad libitum*.

MATERIALS AND METHODS

Animals and housing conditions

The grey mouse lemur (*Microcebus murinus* Miller 1777, Cheirogaleidae) is a small (~80–150 g in captivity) nocturnal primate originating from Madagascar. It feeds on fruits, arthropods, tree gums, insect secretions and small vertebrates (Dammhahn and Kappeler, 2008). In captivity, it lives ~8 years, reproduces at 1 year old and gives birth to ~1–3 offspring per year. Grey mouse lemurs are seasonal breeders. They become sexually active and lose body mass during the long day period (>12 h of light per day, wet season) whereas they gain mass during the resting autumnal period to reach their maximal weight in mid-winter (short day period, dry season) (Perret and Aujard, 2001b). In captivity, seasonal variations of physiological functions are entrained by alternating 6-month periods of long day length [14 h of light per day; long day (LD) period] and short day length [10 h of light per day; short day (SD) period] under artificial light (Génin and Perret, 2000).

Twenty-four adult female (2–4 years old) grey mouse lemurs were obtained from our captive colony (Brunoy, Muséum National d'Histoire Naturelle, France, European Institution Agreement No. 962773), which was founded from a stock originally imported from southern Madagascar in 1967–1972. In the present study, females were kept under the winter-like photoperiod (SD), i.e. when wild animals have to cope with the food and water shortages that characterize the dry season. They were housed individually in cages (50×40×30 cm) to discard the confounding effects of social interactions, with branches and one nest box. They were maintained at a thermoneutral ambient temperature (24–25°C) (Aujard et al., 1998) corresponding to the mid-winter mean temperature of Madagascar (Jury, 2003) and at a relative humidity of 55%. We chose to implement this experiment at thermoneutrality (and not under a simulated, daily cycle of ambient temperature) to remove the confounding effect of thermoregulation on energy-saving adjustments.

Body mass, energy intake and food efficiency

Individuals were fed with a standardized homemade blended mixture of spice bread, egg, concentrated milk, white cheese, baby cereals, mixed fresh banana and water (Giroud et al., 2008). The macronutrient composition of the mixture was 50% carbohydrates, 20% proteins and 30% lipids, with a caloric value of 4.8×10^3 J g^{-1}.

Under captive conditions, SD isolated animals tend to overfeed and overfatten, eventually reaching an abnormal, obese state. Hence, the daily amount of mixture delivered to the so-called '*ad libitum*' individuals was clamped to the level required to secure a normal mass gain (M. Perret, unpublished data) (Giroud et al., 2008). Water was available *ad libitum*.

The chronic treatment lasted 11 weeks during the first half of the SD period. Twelve females were fed *ad libitum* (AL) and 12 received 60% of the amount of food of the AL (CR; Fig. 1). This simulated favorable *versus* harsh dry-season conditions. Afterward, the short-term treatment started and lasted for 12 days. Six females per treatment remained with the same level of food availability (control treatment; AL100, CR60), whereas six others were exposed to an 80% calorie restriction, i.e. they were fed with 20% of the amount of food of the AL100 (acute treatment; AL20, CR20; Fig. 1). Daily calorie intake corresponded to 144×10^3 J for AL individuals, 86.4×10^3 J for CR individuals and 28.8×10^3 J for AL20 and CR20 individuals. Daily feeding took place *ca.* 1 h before lights-off to avoid disturbance of the natural biological rhythm. For administrative reasons, on day 6, animals received twice their usual amount of food, and no food was given on day 7 (Sunday). For the sake of homogeneity in the characterized responses to calorie availability, data from days 6–7 are not included in the present analyses. Food efficiency ($g J^{-1}$) was calculated by dividing the difference in body mass by the total food intake (J) over the 12 days of the short-term treatment (Zabala et al., 2006).

Urinary cortisol excretion

Urinary cortisol excretion was determined to assess whether the energetic response imposed by a caloric stress was induced by a different stress response. If calorie restriction induces a physiological state of chronic stress, the energy consumption due to the stress response could partly confound the energetic effect of calorie shortage. To test if calorie-restricted animals were stressed, we assayed cortisol concentration in the urine. Urine samples (0.25–1 ml) were collected on days 5 and 12, 60–90 min before the dark phase, by spontaneous urination during handling, and were stored at –80°C. Urinary cortisol concentrations were measured in duplicate from 10 µl of urine using an enzyme immunoassay (DE2989, Demeditec Diagnostics GmbH, Kiel-Wellsee, Germany). The sensitivity of this method is 2.5 ng ml^{-1}. The inter- and intra-run coefficients of variation are 7.8 and 7.4%, respectively. To control for variations in diuresis, the concentration of creatinine (µg g^{-1} creatinine) of each sample was measured with an enzyme immunoassay (8009, Metra®Creatinine, Quidel Corporation, San Diego, CA, USA). Body mass was used as covariate in the analysis.

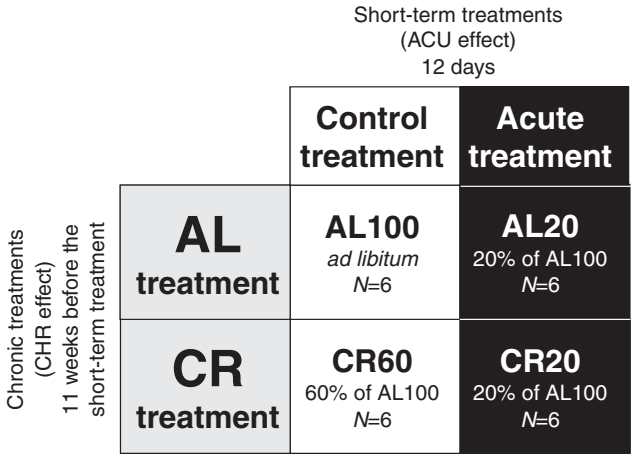

Fig. 1. Experimental design. Each block defines the denomination of the treatment, the daily amount of food provided and the sample size. During the chronic treatment before the short-term treatment, the 24 female grey mouse lemurs were either fed *ad libitum* (*N*=12) or were calorie restricted (60% relative to *ad libitum* individuals, *N*=12). Then, for the experiment, animals were divided into four groups: AL100 and CR60 individuals were fed as during the chronic treatment, hence constituting a control treatment, whereas AL20 and CR20 individuals were fed with 20% of the amount of food provided to *ad libitum* individuals (AL100; acute treatment). A significant CHR effect indicates a difference between the AL and the CR treatments. A significant ACU effect indicates a difference between the control and the acute treatments. A significant CHR×ACU interaction indicates that the response to the acute food shortage (acute treatment) differed according to former food availability (chronic treatments). A greater change through time of the physiological state of CR20 individuals relative to AL20 is indicative of acclimation to food shortage.

Body temperature and locomotor activity parameters

Body temperature and locomotor activity were recorded using telemetry, with internal thermosensitive radio-transmitters (TA10TA-F20, 3.2 g, Data Sciences International, St Paul, MN, USA) and receiver boards (RPC-1, Data Sciences International) placed in each cage. Radio transmitters were implanted into the visceral cavity by surgery under general anaesthesia. Animals were pre-anaesthetized with Valium (10 mg, 2 mg 100 g^{-1} intramuscular injection), and then anesthetized using 5% isoflurane (Forene, Abbott, France) vaporized in an anaesthesia induction chamber. Anaesthesia was maintained throughout the surgery by a 3.5–5% isoflurane gas anaesthesia delivered by a mask placed over the snout. After surgery, individuals received analgesia (0.04 ml 100 g^{-1}, Metacam, Boehringer Ingelheim, Burlington, ON, Canada). Calibration for each transmitter was provided by the manufacturer. The experiment began 14 days after surgery. During this recovery period, animals were fed with their normal calorie-restriction treatment. Body temperature (T_b, ±0.1°C) was recorded for 10 s every 5 min. Locomotor activity (arbitrary activity units, a.u.) was continuously recorded by the receiver from vertical and horizontal movements. The number of movements was summed over periods of 5 min. Daily profiles of locomotor activity were extracted from the raw data with Dataquest Lab Pro ver. 3.0 (Data Sciences International). After the study, transmitters were removed *via* surgery.

Daily profiles of body temperature were characterized using four parameters (see Fig. 2) (Genin and Perret, 2003; Giroud et al., 2008): minimum body temperature ($T_{b,min}$, °C) and its time of occurrence (H_{min}, min), onset of T_b decrease (H_{decr}, min) and torpor bout duration (D_{torp}, in min). A torpid state was defined as T_b<33°C (Ortmann et al., 1997; Genin and Perret, 2003). Times of occurrence preceding lights-on (phase advances) were expressed by negative values (H_{decr}). Inversely, phase delays were expressed by positive values (H_{min}).

Locomotor activity was characterized by four parameters (see Fig. 2): the intensity of locomotor activity during the normothermic state (LA_{norm}, a.u. min^{-1}), measured as the sum of the locomotor activity for the active period divided by the duration of the active period; onset of locomotor activity (min), relative to the time of lights-off; duration of locomotor activity (D_{act}, min), measured as the time between onset and offset; and total amount of locomotor activity (LA_{tot}, a.u.). To account for the fact that increased time spent in torpor mathematically reduces the time of locomotor activity, we needed to assess locomotor activity during the normothermic state. Hence, we identified the threshold in T_b (i.e. under which locomotor

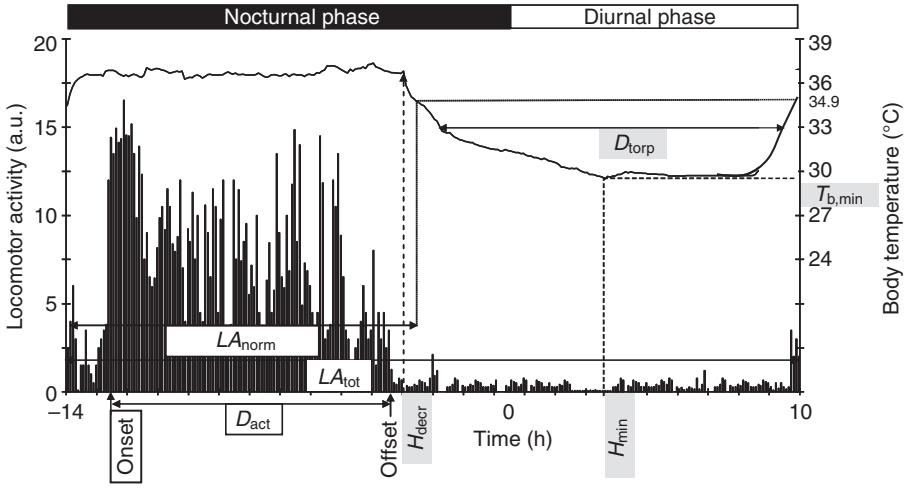

Fig. 2. Parameters of body temperature (grey boxes) and locomotor activity (white boxes) of grey mouse lemurs during the short-day period. The body temperature parameters studied are minimum body temperature ($T_{b,min}$, °C) and its time of occurrence (H_{min}, min), onset of T_b decrease (H_{decr}, min) and torpor bout duration (D_{torp}, min). A body temperature higher than 34.9°C defines the active period during the normothermic state (supplementary material Fig. S1). Four locomotor activity parameters were studied: the intensity of locomotor activity during the normothermic state (LA_{norm}, a.u. min^{-1}), measured as the sum of the locomotor activity for the active period divided by the duration of the active period; the onset of locomotor activity (min), relative to the time of lights-off; the duration of locomotor activity (D_{act}, min), measured as the time between onset and offset; and the total amount of locomotor activity (LA_{tot}, a.u.).

activity rapidly declined) that distinguishes LA_{norm} from residual locomotor activity during hypothermia. This was done with a piecewise regression applied to all available data. The onset and duration of actual locomotor activity were computed with Clocklab (Actimetrics Inc., Evanston, IL, USA). 'Day' expressed in our results began at the onset of the nocturnal phase and lasted 24 h.

Statistical analyses

Results are given as means ± s.d. for all figures but Fig. 4 (s.e.m., for a better readability). $P<0.05$ was considered significant. All data of telemetric parameters were analysed with linear mixed effect models (LME), built with the 'nlme' function (Pinheiro et al., 2005) in R ver. 2.8.1 (R Development Core Team, Vienna, Austria). Normality of models' residuals was checked with normal quantile-quantile plots.

Explanatory variables were the fixed effects of the chronic level of food availability (CHR effect; AL *versus* CR), the level of food availability during the short-term treatment (ACU effect; control treatment *versus* acute treatment), the effect of time (10 days for T_b and locomotor activity parameters, week 1–2 for urinary cortisol values) and all their interactions. Inter-individual variability was accounted for by declaring individual identity and individual response to time as random effects (Schielzeth and Forstmeier, 2009). As we did not know *a priori* the shape of the temporal response of parameters in the different treatments, we identified the most parsimonious structures for the effect of time for each variable using Akaike's information criterion (AIC)-based model selection. Three temporal structures were compared: a linear (days), a logarithmic [log(day)] or a factor [ordered (days)] effect of time. For the sake of comparability among studies, all slopes for the continuous effect of time were estimated with a linear term; slopes are given if *t*-tests indicated significant departure from temporal stability (slope≠0). Increased flexibility of individuals exposed to former food shortage (i.e. acclimation) would be revealed by significant interactions between the chronic and the short-term treatments (CHR×ACU or CHR×ACU×time effects), given that CR20 individuals have a greater change in their physiological state over time than AL20 individuals.

Because we used temporal repeated measures, and because the experimental treatments may have affected inter-individual and intra-individual variability, different variance covariates were used to adjust tests for non-independence of residuals among data points (Anderson et al., 1998). The most parsimonious structures were identified by AIC comparison of alternative modelling of the residual variance (Pinheiro and Bates, 2000). The auto-regressive model of order 1 (Pinheiro and Bates, 2000) was the most parsimonious autocorrelation structure. For heteroscedasticity, the structures that were selected were among-treatment variation (varIdent function) and/or treatment-specific exponential temporal variation in residual variance (varExp function).

The starting model had the following form [notation follows Zuur et al. (Zuur et al., 2009), where i is the index for the individual, j is the index for time (for the description of the autocorrelation parameter, j takes the values s and t) and k is the index for the combination of CHR and ACU effects):

$$Y_{ijk} = \alpha + \beta_1 Time_{ij} + \beta_2 \mathrm{CHR}_i + \beta_3 \mathrm{ACU}_i + \beta_4 Time_{ij} \mathrm{CHR}_i + \beta_5 Time_{ij} \mathrm{ACU}_i + \beta_6 \mathrm{CHR}_i \mathrm{ACU}_i + \beta_7 Time_{ij} \mathrm{CHR}_i \mathrm{ACU}_i + b_{i1} + b_{i2} + \varepsilon_{ijk},$$

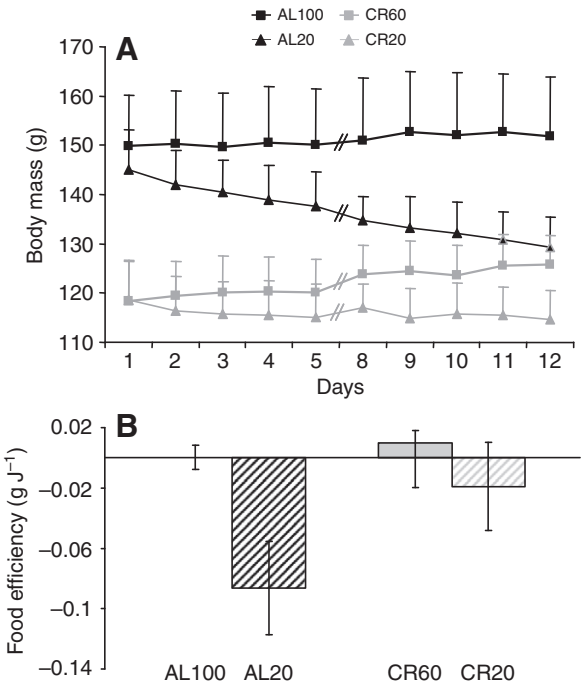

Fig. 3. Effect of food restriction treatments on (A) body mass and (B) food efficiency rate [Δbody mass (g)/food intake (J)]. //, time rupture. Error bars are ±s.d.

with random intercept b_{i1}~N(0,d_{11}^2), random slope for the effect of time b_{i2}~N(0,d_{22}^2), cov(b_{i1},b_{i2})~N(0,d_{12}^2), ε_{ijk}~N(0,$\sigma_k^2 e^{2\delta_{kj}}$) and cor($\varepsilon_{isk}$,$\varepsilon_{itk}$)=$\rho^{|t-s|}$ if $t \neq s$.

Once the most likely structures for heteroscedasticity and time dependence were identified, a full model was built. The final model, containing only significant effects, was obtained by deletion of the non-significant interactions and additive effects from this full model. The *F*-tests of the significance of effects were computed with models derived from the final model.

RESULTS

Body mass and food efficiency

At the start of the short-term treatment, which corresponds to the peak of body fat storage during the SD period in captivity, AL and CR individuals weighed 147.5±6.4 and 118.4±5.4 g, respectively.

The temporal variations of body mass were influenced by both the short-term and the chronic treatments [time×(CHR+ACU) effect; Fig. 3A, Table 1], but with no acclimation effect. Body mass of individuals in the CR treatment remained stable (+0.24±0.21 g day^{-1}, t_{107}=1.1, P=0.3) whereas individuals in the AL treatment decreased in body mass during the 12 days of the experiment (CHR×time effect, –1.06±0.16 g day^{-1}, t_{107}=–6.5, P<0.001; Table 1). Acute calorie restriction elicited a significant decrease in body mass (–0.83±0.21 g day^{-1}, t_{107}=–4.0, P<0.001) whereas control individuals continued to fatten (ACU×time effect, +0.42±0.19 g day^{-1}, t_{107}=2.2, P=0.03; Fig. 3A, Table 1). However, at the end of the short-term treatment, the decrease in body mass for AL20 individuals was three times greater (–10.8% of initial body mass) than for CR20 individuals (–3.4%).

Food efficiency was affected by the acute treatment, with an acclimation effect of the chronic calorie restriction (CHR×ACU effect, $F_{1,20}$=10.0, P<0.01). Food efficiency was the greatest for individuals that had been previously calorie restricted (Fig. 3B).

Urinary cortisol concentrations

Urinary cortisol excretion increased (+1.7±0.3 µg g creatinine^{-1} week^{-1}) between the two weeks of experiment, but was not influenced by calorie restrictions (Table 1, supplementary material Table S1).

Daily torpor

Average 24 h profiles of T_b differed among food availability treatments (Fig. 4A). The onset of T_b drop (H_{decr}) was the most flexible component of torpor; it was rapidly adjusted in response to the acute treatment, and with an acclimation effect (Table 1). Control calorie-restricted individuals (CR60) entered torpor 4.4 h before individuals fed *ad libitum* (AL100; supplementary material Table S1). Former restriction further advanced the drop in T_b (ACU×CHR×time effect; Table 1). CR20 individuals more rapidly advanced their time of body temperature decrease than AL20 individuals relative to their respective controls (Fig. 5A). On average, CR20 individuals begin to decrease their T_b 9.3 h before lights-on, 5.9 h earlier than non-acclimated individuals (AL20, t_{53}=2.38, P=0.04).

Torpor bout duration (D_{torp}) differed across time between controls and acutely restricted individuals (ACU×time effect; Table 1), although it was mainly influenced by the chronic treatment (CHR effect), with no acclimation effect (Fig. 5B).

Table 1. Effects of calorie restriction treatments and time on body temperature, locomotor activity, body mass and urinary cortisol excretion in grey mouse lemurs

Effect	d.f.	*F*	*P*	d.f.	*F*	*P*	d.f.	*F*	*P*	d.f.	*F*	*P*
	H_{decr}			D_{torp}			$T_{b,min}$			H_{min}		
Time	1; 200	7.64	**0.01**	1; 208	11.69	**<0.001**	1; 215	18.75	**<0.001**	1; 205	1.46	0.23
CHR	1; 20	12.68	**<0.01**	1; 21	40.12	**<0.001**	1; 21	23.40	**<0.001**	1; 21	0.12	0.74
ACU	1; 20	2.93	0.10	1; 21	0.81	0.38	1; 21	0.10	0.76	1; 21	0.24	0.63
Time × CHR	1; 198	2.25	0.14	1; 206	0.04	0.84	1; 213	0.19	0.67	1; 203	5.13	**0.02**
Time × ACU	1; 198	2.72	0.10	1; 206	5.18	**0.02**	1; 213	2.75	0.10	1; 203	3.89	0.05
CHR × ACU	1; 19	0.19	0.67	1; 20	1.12	0.30	1; 20	0.32	0.58	1; 20	1.73	0.20
Time × CHR × ACU	1; 197	7.92	**0.01**	1; 205	0.63	0.43	1; 212	0.78	0.38	1; 202	3.87	0.05
	LA_{norm}			Onset			D_{act}			LA_{tot}		
Time	1; 214	1.07	0.30	9; 201	4.52	**<0.001**	1; 205	0.35	0.55	1; 215	6.69	**0.01**
CHR	1; 21	8.77	**0.01**	1; 21	2.84	0.11	1; 21	17.32	**<0.001**	1; 21	1.67	0.20
ACU	1; 21	4.55	**0.04**	1; 21	2.75	0.11	1; 21	2.84	0.11	1; 21	0.04	0.84
Time × CHR	1; 212	0.01	0.93	9; 183	1.96	0.05	1; 203	0.72	0.40	1; 213	2.20	0.14
Time × ACU	1; 212	3.77	0.05	9; 183	2.12	**0.03**	1; 203	0.33	0.56	1; 213	2.32	0.13
CHR × ACU	1; 20	4.99	**0.04**	1; 20	0.91	0.35	1; 20	0.00	0.98	1; 20	0.98	0.34
Time × CHR × ACU	1; 211	0.46	0.50	9; 174	0.75	0.66	1; 202	4.97	**0.03**	1; 212	0.37	0.54
	Body mass			Urinary cortisol excretion								
Time	1; 215	0.66	0.42	1; 19	10.25	**<0.005**						
CHR	1; 21	11.57	**<0.01**	1; 20	0.11	0.75						
ACU	1; 21	0.80	0.38	1; 20	2.52	0.13						
Time × CHR	1; 213	11.86	**<0.001**	1; 17	1.04	0.32						
Time × ACU	1; 213	29.12	**<0.001**	1; 17	0.27	0.61						
CHR × ACU	1; 20	0.25	0.63	1; 19	0.47	0.50						
Time × CHR × ACU	1; 212	2.38	0.12	1; 16	0.00	0.96						

Tests were obtained with linear mixed models. Explanatory variables are the fixed effects of chronic calorie restriction (CHR), acute calorie restriction (ACU) and the time since the start of the short-term treatments. The effect of time was modelled as a logarithm [log(day)] for $T_{b,min}$ and LA_{tot}, as factor [ordered (days)] for onset, as a factorial effect of the week for urinary cortisol excretion and as a linear effect of days for all other variables. Values in bold are significant (P<0.05).

D_{act}, duration of locomotor activity; D_{torp}, torpor bout duration; H_{decr}, onset of T_b decrease; H_{min}, time of minimum body temperature; LA_{norm}, intensity of locomotor activity during the normothermic state; LA_{tot}, total amount of locomotor activity; T_b, body temperature; $T_{b,min}$, minimum body temperature.

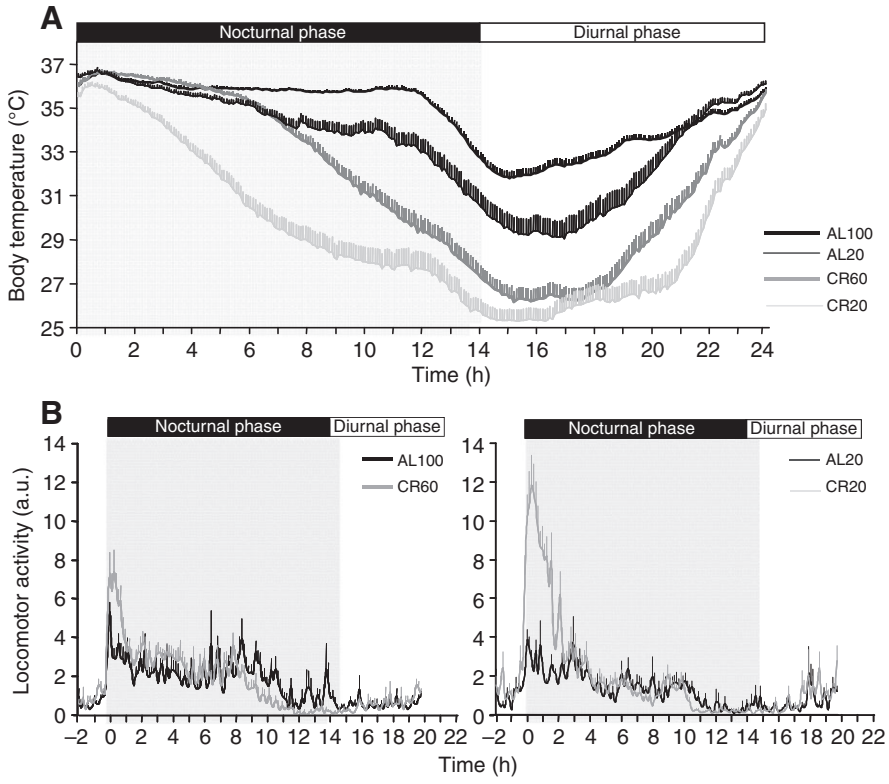

Fig. 4. Daily profiles of (A) body temperature and (B) locomotor activity (±s.e.m.; averaged over 10 days) for each food availability treatment.

On average, AL20 and CR20 individuals spent ~2.9 h more time in torpor than control individuals (acute treatment: 16.9±5.6 min day^{-1}, t_{103}=2.13, P=0.04; control treatment: 2.3±4.3 min day^{-1}, t_{104}=0.53, P=0.59). The acute restriction increased torpor duration by 21.8±10.5 min day^{-1} for AL20 individuals (t_{52}=2.15, P=0.03) and by 14.8±3.9 min day^{-1} for CR20 individuals (t_{51}=3.7, P<0.001), whereas it remained stable for individuals maintained in their former treatment (AL100: 2.7±7.6 min day^{-1}, t_{51}=0.35, P=0.7; CR60: 3.2±4.4 min day^{-1}, t_{53}=0.71, P=0.5). CR individuals spent ~8.8 h more time in torpor than AL individuals (effect CHR; supplementary material Table S1).

Minimum body temperature ($T_{b,min}$) was only adjusted in the long-term, i.e. in response to the chronic treatment (CHR+time effect; Table 1). Torpor depth was greater in the CR than in the AL treatment (Fig. 5C). On day 12, AL individuals reached body temperatures of 29.4±1.4°C whereas CR individuals decreased their body temperature to 24.3±0.7°C, corresponding to the ambient temperature (supplementary material Table S1). The hour of maximal torpor depth (H_{min}) was also adjusted in the long term (CHR×time effect; Table 1), but was nearly insensitive to the acute restriction (including the ACU effect; Table 1, Fig. 5D). The CR treatment induced a delay of 5.8±2.7 min day^{-1} (t_{104}=2.1, P=0.03) to reach the minimum body temperature whereas H_{min} was stable for individuals in the AL treatment (–3.6±2.3 min day^{-1}, t_{103}=–1.6, P=0.12; supplementary material Table S1).

Locomotor activity

Locomotor activity patterns differed according to calorie treatment (Fig. 4B). The threshold of body temperature that distinguishes locomotor activity during normothermia from residual locomotor activity during hypothermia was 34.9°C, regardless of calorie treatment (supplementary material Fig. S1). Intensity of locomotor activity during normothermia (LA_{norm}) increased in response to the acute treatment with an acclimation effect (CHR×ACU effect, Table 1). LA_{norm} values of CR20 and AL20 individuals were 2.7 and 1.3 higher, respectively, in comparison with their respective control individuals (Fig. 4B, supplementary material Table S1). The acclimation effect appeared to be strong given that previously restricted individuals (CR20) doubled the intensity of their locomotor activity (Fig. 5E).

The onset of locomotor activity was flexible, being adjusted in response to both short-term and chronic treatment [time×(CHR+ACU) effect; Fig. 5F, Table 1], but with no acclimation effect. The onset of locomotor activity occurred earlier for chronically calorie-restricted individuals than for AL individuals (Fig. 5F, supplementary material Table S1). Acutely restricted individuals tended to advance their onset (–2.9±1.7 min day^{-1}, t_{102}=–1.8, P=0.07) whereas it remained stable for control individuals (–1.03±1.6 min day^{-1}, t_{104}=–0.6, P=0.5).

The duration of the active period (D_{act}) was mainly adjusted in the long term with a major effect of the chronic treatment (CHR effect; Table 1). CR individuals were active 4 h less than AL individuals (supplementary material Table S1). However, the weak interaction effect between both treatments and time (time×CHR×ACU effect; Table 1) suggests that the duration of activity was flexible. In response to the acute calorie restriction, AL20 and CR20 individuals reduced the length of their active period by 14 and 22%, respectively (Fig. 5G).

Although acutely restricted animals were hyperactive in the first hours of night, the total amount of locomotor activity was similar between food availability treatments (effects including CHR and/or ACU; Table 1, supplementary material Table S1). Total locomotor activity decreased by 7.1±3.8 a.u. day^{-1} (time effect; Table 1, Fig. 5H).

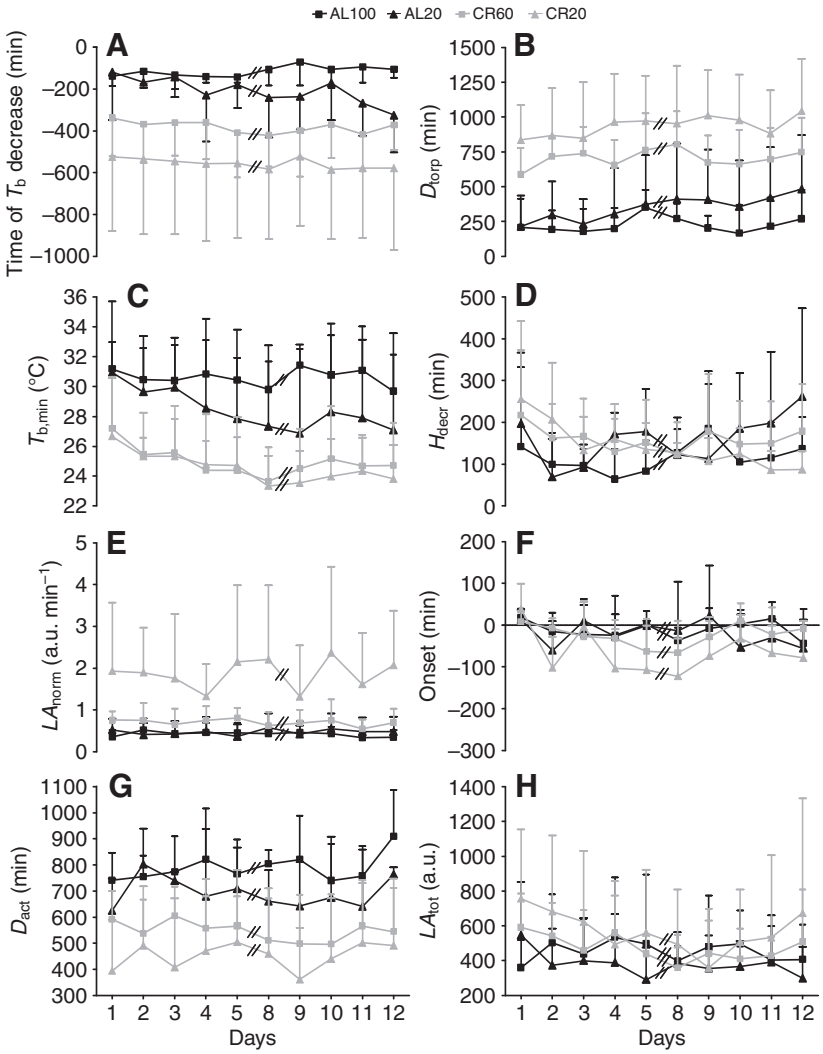

Fig. 5. Effect of food restriction treatments over the 12 days of the short-term treatment on (A) the time of T_b decrease, (B) D_{torp}, (C) $T_{b,min}$, (D) H_{decr}, (E) LA_{norm}, (F) onset, (G) D_{act} and (H) LA_{tot}. Values are means ± s.d. Variables are defined in Fig. 2. //, time rupture.

DISCUSSION

Heterothermic organisms overcome seasonal energetic constraints by plastic physiological changes that reduce their energy expenditure, such as daily torpor and inactivity (Ruf, 1991; Boyles et al., 2007; Stawski and Geiser, 2010). However, to date no study has determined to what extent these organisms can flexibly adjust these energy-saving mechanisms according to unexpected energetic constraints: can they launch them rapidly, or only in the long term? And, is this physiological change facilitated by former exposure to a mild, energetic shortage (i.e. acclimation)? We answered these questions by quantifying the physiological adjustments of a small, heterothermic primate exposed to contrasted regimes of food shortage.

Torpor use was highly flexible, being increased in both the short term and long term in response to food shortage. However, the different components of torpor were not equally flexible. The most flexible were the hour of start of body temperature decrease and torpor duration. The flexibility of the timing of minimum body temperature and torpor depth were limited: animals did not change the onset nor decrease their minimum body temperature in response to the acute food shortage, and CR individuals were at the lowest body temperature they could achieve, i.e. ambient temperature. As flexibility of torpor could be evidenced at a constant ambient temperature of 25°C, in the field, the energetic constraints added by a lower ambient temperature would allow the expression of an even greater flexibility. In nature, grey mouse lemurs reach body temperatures as low as 11.3±1.8°C for an ambient temperature of 9.4°C (Schmid, 2000). Also, in the field, arousal should be helped by rising ambient temperature during the day, with a passive rewarming of torpid animals (Turbill et al., 2008; Schmid, 2000). Such energetic benefits driven by ambient temperature variations were prevented in our experimental design. Lastly, captive animals may have a decreased ability to use torpor. Reduced torpor was observed after two to four generations of captivity only in feathertail gliders (Geiser and Ferguson, 2001) and hamsters (Chaffee, 1966). Overall, it suggests that torpor flexibility should be even greater in nature than what we found with captive-bred animals.

Rapid adjustments of torpor use in response to acute food shortages had already been demonstrated in captive mouse lemurs. The onset of body temperature decrease was advanced by 1 h in response to 3 days of fasting (Séguy and Perret, 2005) and by 4 h in response to 8 days of 80% food restriction (similar to the AL20 treatment) (Genin and Perret, 2003). The torpor depth achieved by acutely calorie-restricted individuals was of the same order of magnitude as in our experiment: ~27.5°C after 2–4 days for individuals 80% food-restricted (Genin and Perret, 2003), and after 12 days for males 40 or 80% food-restricted (Giroud et al., 2008). In comparison with these data, our results highlight that torpor depth

can be further increased by exposure to a chronic moderate calorie restriction. Torpor duration was also flexible in the short term, increasing by 5 h during 14 days of food restriction (whatever the intensity of the restriction) (Giroud et al., 2008). Torpor patterns also vary in the field. The duration of torpor bouts ranged from 3.6 to 18.2 h, mainly by an advance of torpor onset, as the end of torpor was tied to a narrow window close to the maxima of sun radiation (4–5 h prior to sunset) (Ortmann et al., 1997; Schmid, 2000). In several species, enhancement of torpor use has been observed in response to natural food shortage. In the Chilean mouse oppossum (*Thylamys elegans*), daily energy requirement was correlated to minimum T_b, torpor frequency, bout and depth (Bozinovic et al., 2007). Higher frequency of torpor was also induced by lesser food availability in elephant shrews (*Elephantulus* spp.) (Lovegrove et al., 2001) and eastern chipmunks (*Tamia striatus*) (Humphries et al., 2003).

Modulations of locomotor activity did not support the hypothesis of energy savings through reduction of muscular activity. Total locomotor activity was not reduced. Nonetheless, the temporal pattern of locomotor activity was highly flexible. A chronic food restriction induced a 30% reduction of the period of activity, a value close to the 49% reduction found by Giroud et al. (Giroud et al., 2008) in males. Furthermore, acutely restricted individuals decreased their active period by 2 h relative to individuals in the control treatment. In addition, the intensity of the locomotor activity of acutely restricted individuals was increased in both the short-term and chronic treatments. Starvation-induced hyperactivity in the first hour of night has already been observed in mouse lemurs (Genin and Perret, 2003; Giroud et al., 2008) and in other small vertebrates (Cornish and Mrosovsky, 1965; Sherwin, 1998). Under natural conditions, intense foraging or migratory behavior would increase the chances of survival of free-ranging animals (Gutman et al., 2007).

In the present study, acclimation to food shortage increased the flexibility of energy-saving mechanisms at four levels. Acclimation increased the energy savings gained by flexibility of the onset of body temperature drop. Moreover, food efficiency increased significantly, reducing body mass loss. Finally, chronically restricted animals were the only ones to both strongly increase their level of activity just after torpor arousal and decrease their active period. If this physical hyperactivity is advantageous, acclimation would improve the beneficial effects of plastic modulation of locomotor activity. The beneficial effects of acclimation are well known for abiotic constraints, such as temperature stresses (Hoffmann et al., 2003; McKechnie and Wolf, 2004; Seebacher, 2005; Bowler and Terblanche, 2008), but have been rarely addressed in the context of the physiological response to food shortage (Ostrowski et al., 2006; Zhao and Wang, 2009). Our results are the first to suggest that former presentation to low food availability increases the ability of heterothermic organisms to overcome unpredicted, drastic food shortages.

Torpor flexibility had some obvious limits. First, even if torpor provided energy savings, it compensated only for part of the 80% energetic shortage as acutely restricted individuals lost body mass (e.g. Giroud et al., 2008). Hence, an 80% food restriction would be close to the maximum energetic shortage that can be compensated for by physiological flexibility. Second, the timing of maximal torpor depth was narrowed to lights-on (e.g. Séguy and Perret, 2005). In fact, some components of the architecture of torpor are shaped by the circadian rhythm (Perret and Aujard, 2001a). The fixed time of the start of the return to normothermia is one major limit of the flexibility of torpor. In captivity, all grey mouse lemurs return to normothermia each day after their torpor episode. This limit would be set by the physiological costs of torpor (increase in oxidative stress, sleep debt, predation risk enhancement) (Humphries et al., 2003; Munro and Thomas, 2004; Giroud et al., 2009; Kokurewicz, 2004). For torpor flexibility to be adaptive, fitness must remain stable despite temporary food shortage. Hence, the fitness consequences of the trade-off between the benefits (energy savings) and the physiological costs of torpor need to be quantified.

The high physiological flexibility of grey mouse lemurs makes sense in an ecological context. High plasticity is supposed to have evolved in response to the unpredictability and low primary production of their native environment (Wright, 1999; Dewar and Richard, 2007). The high physiological flexibility of heterotherms could be a major determinant of their success in overcoming the energetic challenges imposed by climate changes (Geiser and Turbill, 2009; Liow et al., 2009). It would allow them to maintain a positive energy balance despite unfavourable biotic environmental variations. Extreme phenotypic plasticity is common among vertebrates that have evolved in unstable, energetically restrictive environments, such as those exposed to ENSO climatic anomalies (Canale and Henry, 2010). Under natural conditions, grey mouse lemurs are faced with harsher energetic constraints than in the laboratory; wild animals weigh 40% less than captive ones in the mid-winter (Lahann et al., 2006), food availability is lower and foraging is less efficient and exposes starving animals to predation. The lack of chronic stress (cf. constant urinary cortisol excretion) in food-restricted individuals is congruent with an adaptation to limited food availability. The observed phenotypic changes would not be the expression of the launching of an emergency life history stage in response to caloric stress (Wingfield, 2003). Overall, it suggests that wild grey mouse lemurs may exhibit even greater levels of physiological flexibility, associated with increased energy savings and decreased torpor costs. In the wild, torpid animals are passively re-heated by the rise of ambient temperature, and gregarious nesting provides additional energy savings (Schmid, 2000; Séguy and Perret, 2005). It should minimize the production of oxidative stress and the energetic cost for torpor arousal (Gilbert et al., 2010).

We suggest that physiological flexibility of energy-saving mechanisms is a key adaptation to respond to increased climate instability. Ongoing climate changes include an increase of the frequency and intensity of extreme climatic events, particularly in the tropics. Our results demonstrated that food scarcity can be compensated for, at least partially, by the launching of energy-saving mechanisms. More interestingly, if an extreme event occurs during the austral winter, the natural food shortage imposed by the dry season should facilitate the energetic compensation through increased torpor. This is demonstrated by the significant acclimation effect. More generally, the physiological flexibility of grey mouse lemurs is likely to play a role in their phenotypic response to a changing climate. In Madagascar, they could be exposed to an increased occurrence of severe droughts and devastating cyclones. As deforestation has claimed 90% of the island's natural forests (Ingram and Dawson, 2005), forest fragmentation will limit distributional range shifts. Hence, local resilience to climate change within the remaining forest fragments is likely to be the only solution for many organisms. High physiological flexibility seems to be an important mechanism to take into account when predicting the ability of organisms to overcome both habitat and climatic changes (Ghalambor et al., 2007; Chown and Gaston, 2008). Future work should put emphasis on the limits and costs that compromise the adaptiveness of physiological plasticity.

LIST OF ABBREVIATIONS

AIC	Akaike's information criterion
AL20	individuals previously fed *ad libitum* that received 20% of the *ad libitum* ration
AL100	control individuals fed *ad libitum*
CR20	individuals previously 40% calorie restricted that received 20% of the *ad libitum* ration
CR60	individuals that received 60% of the *ad libitum* ration
D_{act}	duration of locomotor activity
D_{torp}	torpor bout duration
ENSO	El Niño Southern Oscillation
H_{decr}	onset of T_b decrease
H_{min}	time of minimum body temperature
LA_{norm}	intensity of locomotor activity during the normothermic state
LA_{tot}	total amount of locomotor activity
LD	long day
SD	short day
T_b	body temperature
$T_{b,min}$	minimum body temperature

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